IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Yakhini, et al.

Serial No.: 09/921,406

Filed: August 2, 2001

For: Classifying Cancers

Confirmation No.: 6019

Group Art Unit: 1642

Examiner: Gary B. Nickol

Docket No. 10010313-1 (50112-1970)

DECLARATION OF ZOHAR H. YAKHINI
PURSUANT TO 37 C.F.R. §1.131

Commissioner of Patents
Alexandria, VA 22313-1450

Sir,

I, Zohar H. Yakhini, hereby declare that:

1) The invention embodied in the above-identified patent application is directed to methods for classifying cancers.

2) I am advised that the United States Patent and Trademark Office has rejected one or more claims presently pending in the above-identified patent application based, at least in part, upon Bittner, et al. *Molecular classification of cutaneous malignant melanoma by gene expression*. Nature; Vol. 46 pp. 536-40; 3 August 2000 (herein, *Bittner)*. I am further advised that the effective priority date of the *Bittner* publication is August 3, 2000.

3) The invention, however, as embodied in the claims of the present invention was completed by me and my co-inventors Amir Ben-Dor, Michael Bittner, Paul Metzler, Yidong Chen, Nicholas M. Sampas, and Michael E. Dougherty in this country prior to August 3, 2000. Specifically, the invention was "completed" by virtue of reduction to practice prior to the

August 3, 2000 priority date of the *Bittner* publication.

4) As evidence that the present invention was so characterized by reduction to practice, Exhibit "A" is attached hereto.

5) Exhibit "A" is a copy of an email sent from co-inventor Michael Bittner to co-inventors of the present application that has attached thereto a draft manuscript of the *Bittner* reference. The manuscript attached to the email of Exhibit A is in substantially the same form as the published manuscript. The email of Exhibit A predates the *Bittner* reference, and includes details of the methods described in part or in whole at least in independent claims 1, 4, 26, and 28 (with like features contained in respective dependent claims as well). Dates and sensitive information (*e.g.*, email addresses) have been redacted in the document in accordance with applicable USPTO rules.

6) The actual manuscript attached to the email of the Exhibit A is not included in Exhibit A because it is not in an electronic form that can be accessed by the Applicants at this time. The co-inventor Michael Bittner is no longer employed by the National Institutes of Health, and therefore is not able to access the electronic records that contain the manuscript.

7) As further evidence that the present invention was reduced to practice prior to publication of the *Bittner* reference, Exhibit "B" is attached hereto.

8) Exhibit "B" is a copy of the PowerPoint® presentation that I developed along with at least one of my co-inventors. The PowerPoint® presentation predates the *Bittner* reference, and includes details of the methods described in part or in whole at least in

independent claims 1, 4, 26, and 28 (with like features contained in respective dependent claims as well). In particular, the graph of the gene expression patterns on Slide 5 of the document is identical or substantially similar to FIG. 2B of the instant patent application that identifies genes that discriminate melanoma clusters, including the top 22 genes obtained by multi-dimensional scaling (MDS) analysis ranking genes according to their impact on minimizing cluster volume and maximizing center-to-center inter-cluster distance. In addition, the MDS graph depicted on slide 4 of the document is identical or substantially similar to FIG. 1B of the instant patent application, that shows the clustering of gene expression data, and indicates in particular a MDS three-dimensional plot of all 31 cutaneous melanoma samples showing major clustering of 19 samples, and the remaining 12 outlying samples. One skilled in the art would be able to arrive at the method of claim 1 by reviewing the data and suggestions of the PowerPoint® presentation of Exhibit B.

9) Upon information and belief, the Examiner is relying on similar data from the *Bittner* reference as that shown in the document of Exhibit B to reject the instant claims. The inventors of the present application developed the subject matter of the *Bittner* reference prior to the publication date of the *Bittner* reference.

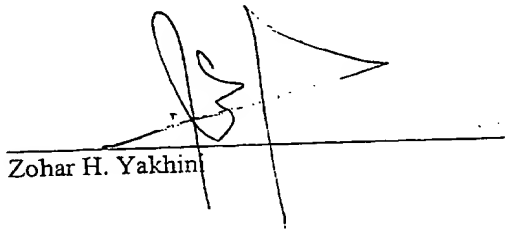
10) As additional evidence that the present invention was reduced to practice prior to publication of the *Bittner* reference, Exhibit "C" is attached hereto.

11) Exhibit "C" is a copy of the invention disclosure submitted to the legal department of Agilent Technologies, the Assignee of record. The invention disclosure includes details of the methods described in part or in whole at least in independent claims 1, 4, 26, and 28 (with like features contained in respective dependent claims as well). In particular, the invention

disclosure discusses on page 1 a method of diagnosing an aggressive form of cancer, based on evaluating expression of WNT5A gene. On page 2 of the invention disclosure, the inventors indicate that the WNT5A's role in melanoma metastasis was suggested in the conclusions of the *Bittner* reference. Therefore, because the inventors are all listed as authors on the *Bittner* reference, the inventors reduced the invention to practice prior to the publication date of the *Bittner* reference. Dates have been redacted in the document in accordance with applicable USPTO rules.

I hereby declare that all statements made herein are of my own knowledge are true and that all statements are made on information and belief and are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

18. Sep. 2005
Date



Zohar H. Yakhini

Lee, Cynthia

From: [redacted]
Sent: [redacted]
To: [redacted]
Cc: [redacted]
Subject: manuscript

Melanoma_MS.pdf.sit

Hi All,

Appended is the revised version of the melanoma manuscript. A few of the math symbols got killed when I put this into pdf format, but they are right in the submitted ms.

Best,
Mike

This file was compressed with Aladdin Systems Stuffit. A free decompression tool, Aladdin Expander for either Windows or Mac may be obtained from the company's internet site at:
http://www.aladdinsys.com/downloads/index.html

EXHIBIT A
PAGE 1 OF 1

Pharma and Diagnostics Applications of DNA Arrays

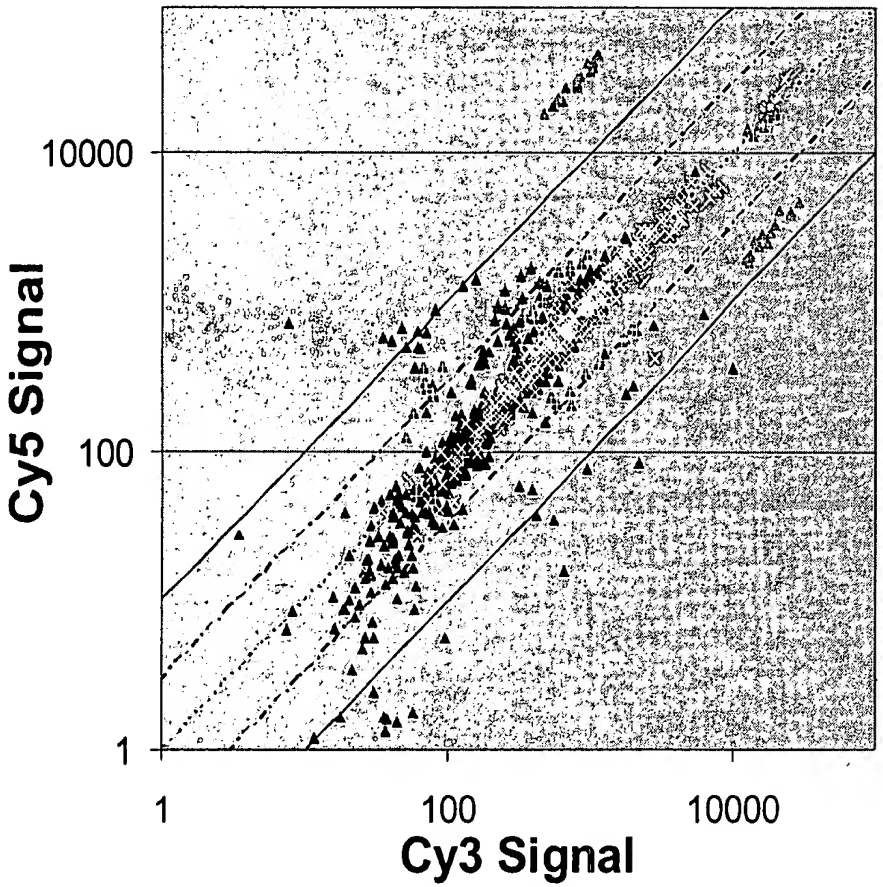
Cancer Diagnostics

- Cancer Classification
  AML vs. ALL (Lander Lab, Whitehead Inst.)

- Cancer Class Discovery
  Melanoma (Trent Lab, NHGRI and Agilent Labs)

Cardiovascular Disease Gene Discovery and Characterization
Quertermous Lab, Stanford and Agilent Labs

cDNA Array Model System Quantitative Results



Lower Limit of Detection
0.5 pM spiked RNA (~3 copies / cell)

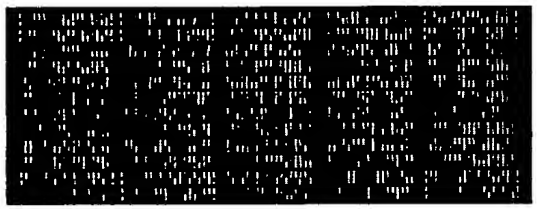
Dynamic Range
0.5 to 50 pM spiked RNA

- Housekeeping Genes
- Negative Controls
- Spiked Controls
- MMS Induction Test Genes
- Waf1
- GOS8
- c-myc
- ATF3
- MAD
- Ratio of 10
- Ratio of 3
- Ratio of 1
- Ratio of 0.3
- Ratio of 0.1

EXHIBIT B
PAGE 2 OF 9

Cancer Classification

Lander Lab, Whitehead Inst., Science Oct. 1999



Leukemia samples - AML vs. ALL
(acute myeloid vs acute lymphoid)

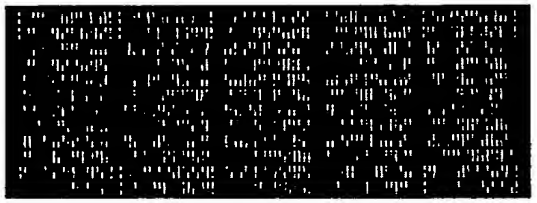
Clustering gene expression patterns of known samples

Diagnostic algorithm using ~100 genes

38 samples diagnosed correctly
1 sample not diagnosed

Molecular Classification of Cutaneous Malignant Melanoma by Gene Expression Profiling

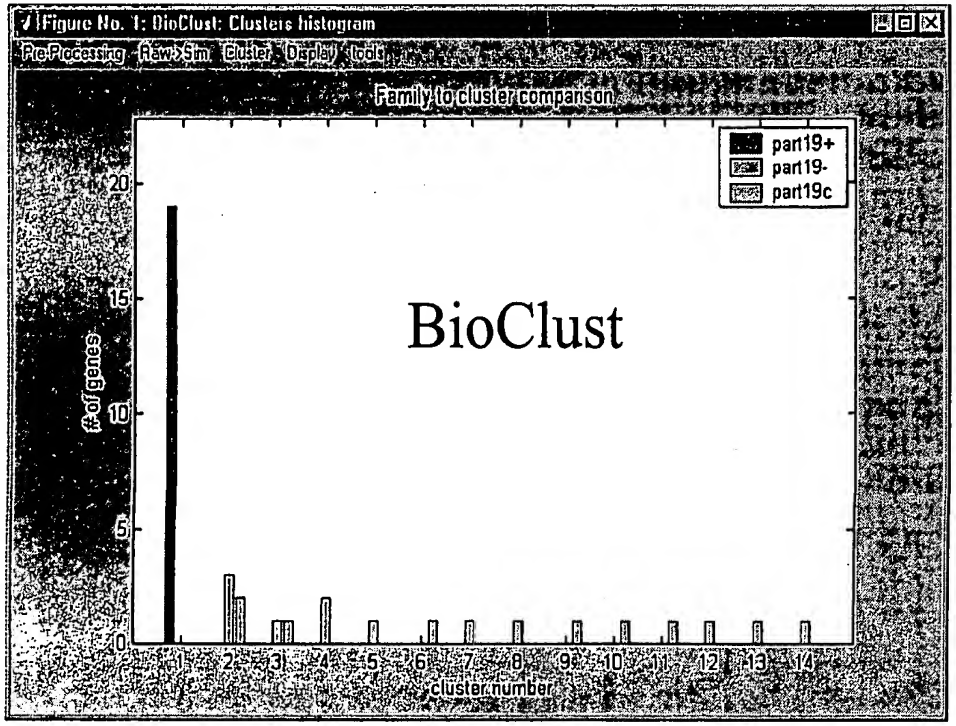
Jeff Trent Lab (NHGRI) et al. and Agilent Labs

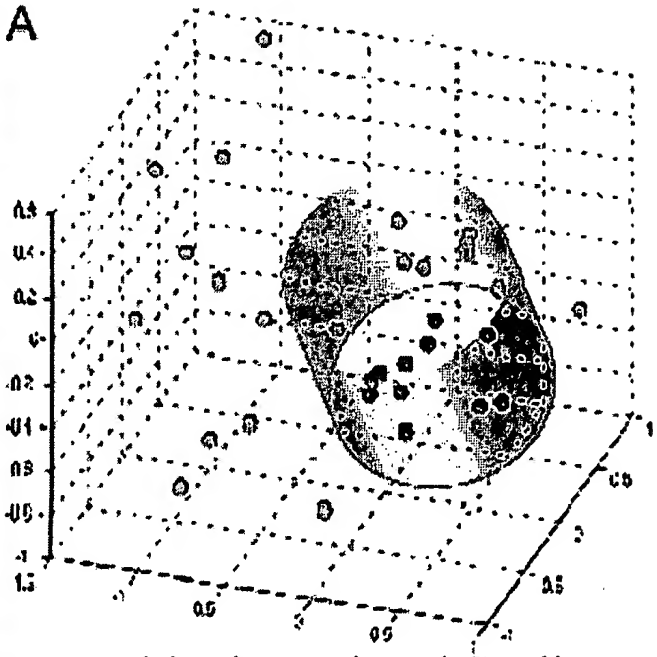


31 melanoma samples
7 control samples

clustering by sample →

Cluster 19
other 12 samples





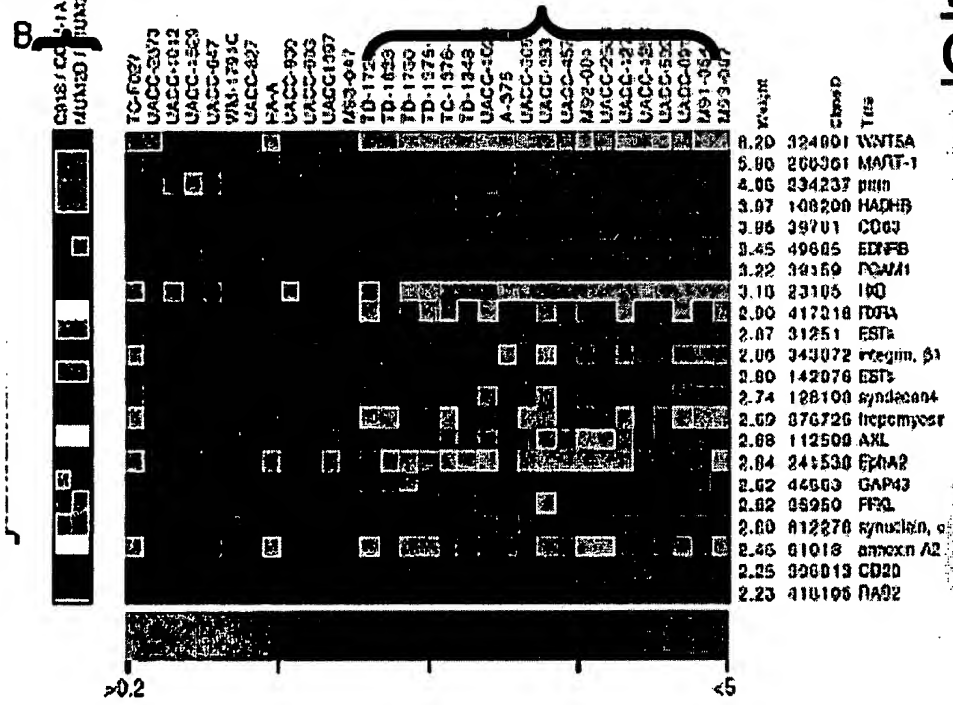
Multi Dimensional Scaling

EXHIBIT B
PAGE 4 OF 9

Biological and Diagnostic Implications

Uveal Melanoma

Cutaneous Melanoma



Weighted List of Cluster 19 Discriminator Genes

- Opposite expression pattern in uveal melanoma samples
- Includes genes with known functions in cell invasiveness
- Cluster 19 melanoma cells less invasive compared to uveal melanoma cells

Gene Expression Patterns as Predictors of Prognosis

Patient Type	# deaths
Cluster 19	3/10
Non-Cluster 19	4/5

(??? statistical significance)

Future joint Agilent/NHGRI experiments to examine statistically significant number of melanoma samples

EXHIBIT B
PAGE 5 OF 9

Cardiovascular Disease

Tom Quertermous et al., Stanford
"Genetic Epidemiology of Atherosclerotic Heart Disease: Advanced Strategies for Prevention and Therapy"

Candidate Gene Library Isolation (SSH) in vitro model systems
- •endothelial
- •smooth muscle
- •macrophages

cDNA Array Expression Profiling in vitro and in vivo systems
- •validate
- •characterize
- •prioritize

Functional Characterization
- •molecular
- •cellular

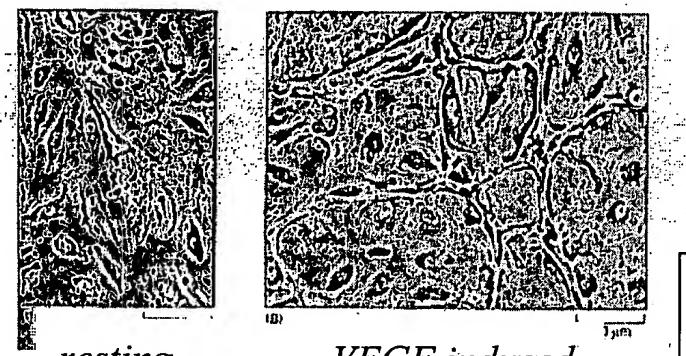
Genotyping Association Studies
~80 candidate genes
~5 SNPs per gene
genotype ~4000 patients

cDNA arrays for Cardiovascular Disease Genes

Agilent Laboratories and Tom Quertermous Lab (Stanford Cardiology)

Angiogenesis

endothelial cell in vitro model system

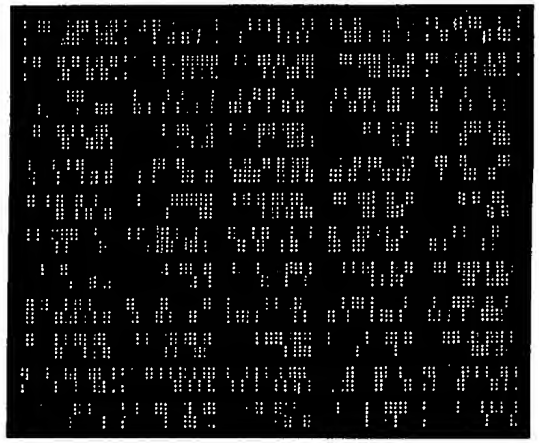


resting *VEGF induced*

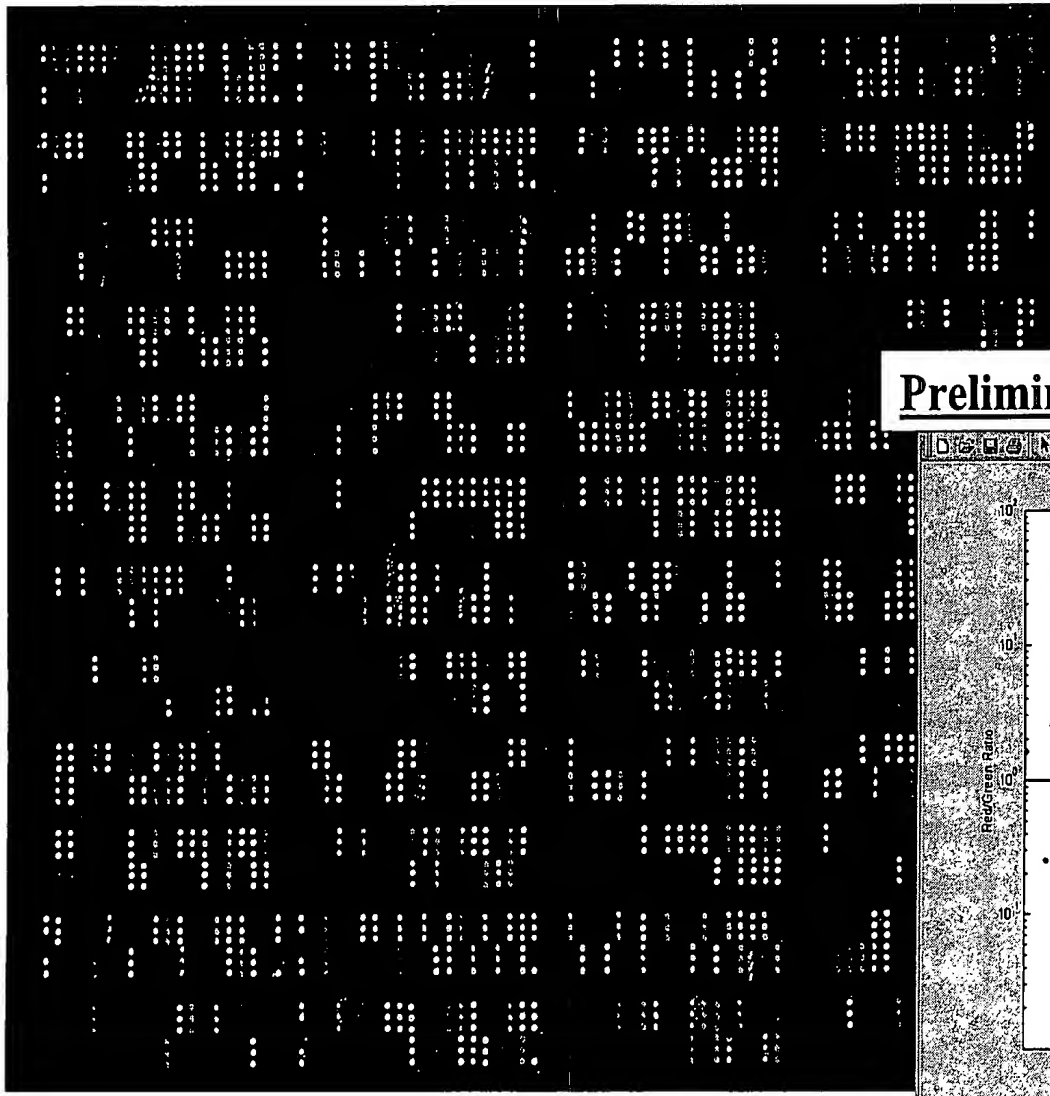
1200 candidate genes up-regulated as endothelial cells begin angiogenesis

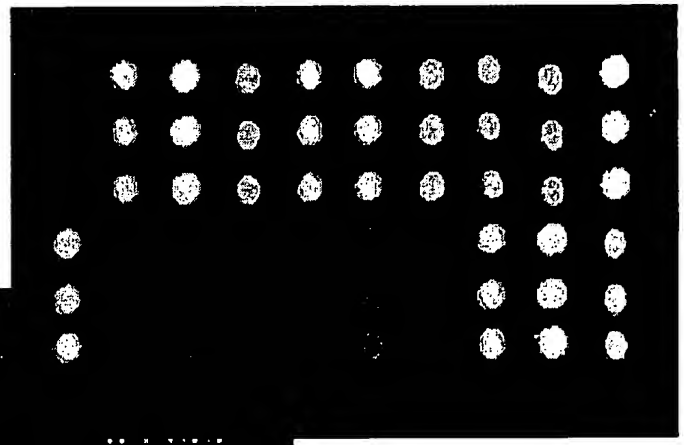
Angiogenesis cDNA Array

- validate angiogenesis clone set
- further characterize gene expression profiles e.g. time course during angiogenesis



Slide 032000_29
Stanford 1200 spotted 03-15-00
Hybed with **Cy3 25ug VEGF ind HUVEC**
+ Cy5 25 ug resting HUVEC





Preliminary L7 Feature Extraction Results

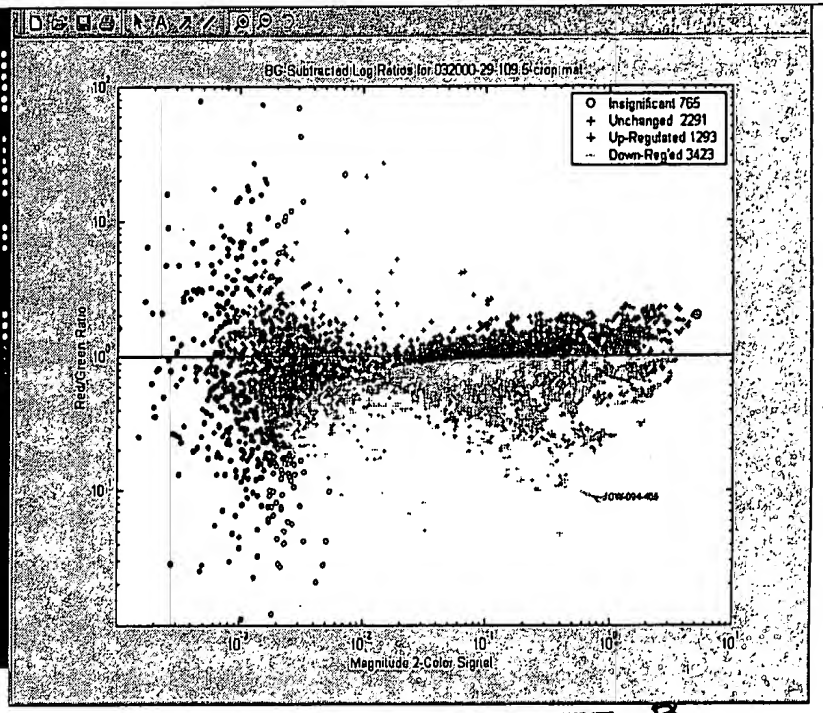


EXHIBIT B
PAGE 8 OF 9

Cancer Classification Results

- Gene expression profile data carry cancer diagnostic information.
- A relatively small subset of genes can be sufficient for tissue classification.
- Other Related Results:
  - Trent Lab (NHGRI)
    melanoma classes
    8000 genes, ~40 samples
  - Lander Lab (Whitehead Inst.)
    leukemias - AML and ALL

00112-1970

Agilent Technologies

INVENTION DISCLOSURE

PAGE ONE OF ____

PDNO 10010313 DATE RCVD [redacted] ATTORNEY MJB/AML

Instructions: The information contained in this document is COMPANY CONFIDENTIAL and may not be disclosed to others without prior authorization. Submit this disclosure to the Agilent Technologies Legal Department as soon as possible. No patent protection is possible until a patent application is authorized, prepared, and submitted to the Government.

Descriptive Title of Invention:

Melanoma Metastasis Inhibition Treatment, Monitoring and Diagnostic Targets.

Name of Project:

Product Name or Number:

Was a description of the invention published, or are you planning to publish? If so, the date(s) and publication(s):

WNT5A was published as ~~an~~ a melanoma informative gene. Causal effect was not published.

Was a product including the invention announced, offered for sale, sold, or is such activity proposed? If so, the date(s) and location(s):

NO

Was the invention disclosed to anyone outside of AGILENT TECHNOLOGIES, or will such disclosure occur? If so, the date(s) and name(s):

Yes. This is a collaboration with NHGRI.

If any of the above situations will occur within 3 months, call your IP attorney or the Legal Department now at 1-650-3061 or 408-553-3061.

Was the invention described in a lab book or other record? If so, please identify (lab book #, etc.)

Not in a lab book. Yes in output of analysis software.

Was the invention built or tested? If so, the date:

No.

Was this invention made under a government contract? If so, the agency and contract number:

Under a CRADA with NHGRI

Description of Invention: *Please preserve all records of the invention and attach additional pages for the following. Each additional page should be signed and dated by the inventor(s) and witness(es).*

A. Prior solutions and their disadvantages (if available, attach copies of product literature, technical articles, patents, etc.).
B. Problems solved by the invention.
C. Advantages of the invention over what has been done before.
D. Description of the construction and operation of the invention (include appropriate schematic, block, & timing diagrams; drawings; samples; graphs; flowcharts; computer listings; test results; etc.)

Signature of Inventor(s): I (we) hereby submit this disclosure on this date: [illegible]

Employee No.	Name	Signature	Telnet	Mailstop	Entity & Lab Name
[redacted]	Zohar Yakhini				
[redacted]	Amir Ben-Dor				

(If more than four inventors, include additional information on another copy of this form and attach to this document)

Form 3.1 IDF1016.DOC Rev. 10/28/00

EXHIBIT C
PAGE 1 OF 6

[redacted]

Melanoma Metastasis Inhibition Treatment, Monitoring and Diagnostic Targets

Invention Disclosure

Amir Ben-Dor
Zohar Yakhini

Background

Using microarray technology to measure the expression profiles of some 3000 human genes in melanoma samples, a putative subclass of melanoma was discovered. The expression characteristics of this subtype correlate well with expression characteristics of a less aggressive type of uveal melanoma. Details of this study are in Bittner et al, Nature, August 2000. Genes that are underexpressed in this putative subtype are expected to be related to the metastasis and progress process of the disease. Some of the more differentially expressed amongst these constitute potential disease stage indicators and treatment targets.

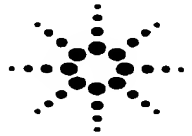
The Invention

WNT5A is one of the genes overexpressed in the more mobile melanoma samples. It is sharply differentially expressed, as indicated by its relevance scores (attached figures show lists of scores). It is also narrowly expressed in the stable class and more spread in the mobile samples, as indicated by the IQRdiff score. The role of WNT5A in melanoma metastasis is further validated by direct measurements of the difference in extracellular matrix penetration, testing samples with low natural WNT5A against the same samples with WNT5A induced expression and against high natural WNT5A samples.

While the sharp differential expression of WNT5A suggests it is useful as an indicator of the disease state, the direct validation goes further to suggest it is an independent cause of the increased mobility of the cells and therefore a potential therapeutic target. Possible therapeutic approaches will be anti-sense or other inhibition mechanisms, keeping WNT5A at low levels.

Claims outline

- WNT5A as a component of a melanoma staging or diagnostic assay.
- WNT5A as a target for melanoma therapeutics.
- WNT5A as a component of therapies or diagnostic approaches to other cancers or other disease where increased cell mobility can accelerate progress.
- Other genes from this study as components of potential melanoma diagnostic and therapeutic approaches.



Prior Art

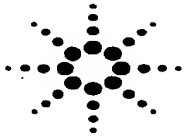
As indicated above, WNT5A's role in melanoma metastasis was suggested in the conclusions of the study published in Nature, August 2000. It is one of many genes listed there as related to melanoma clinical behaviour. Its role as a cause for increased cell mobility is validated in an unpublished NHGRI study.

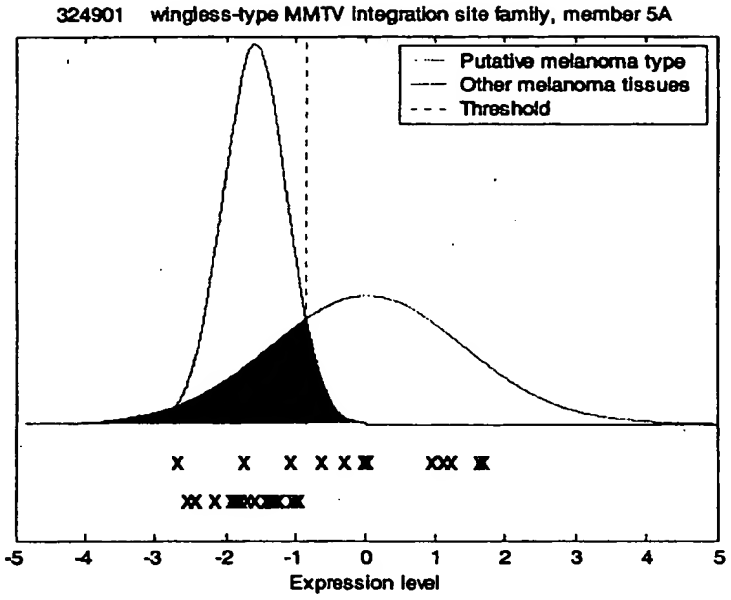
Additional Documents

An invention disclosure submitted by Bittner et al, at the NIH. These should be combined to a single co-invention

Inventorship

From Agilent Labs: Amir Ben-Dor, Zohar Yakhini.
From NHGRI: as listed in their disclosure.





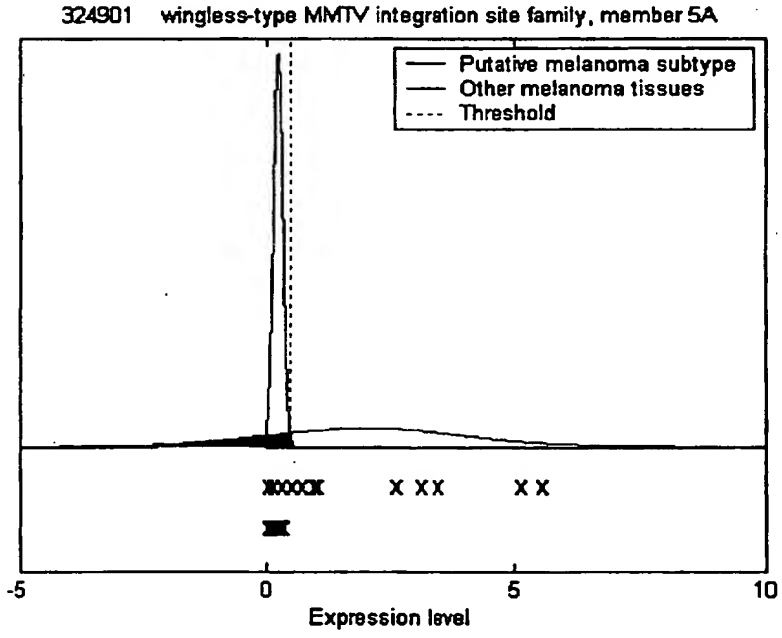


Figure 1. Expression levels of WNT5A, for the two types of melanoma suggested in Bittner et al, 2000. Actual numbers in the top plot are log ratios and in the bottom plot the actual ratios. Gaussian fits for each class are depicted. Gaussian distribution assumption is better founded for the log ratio values.

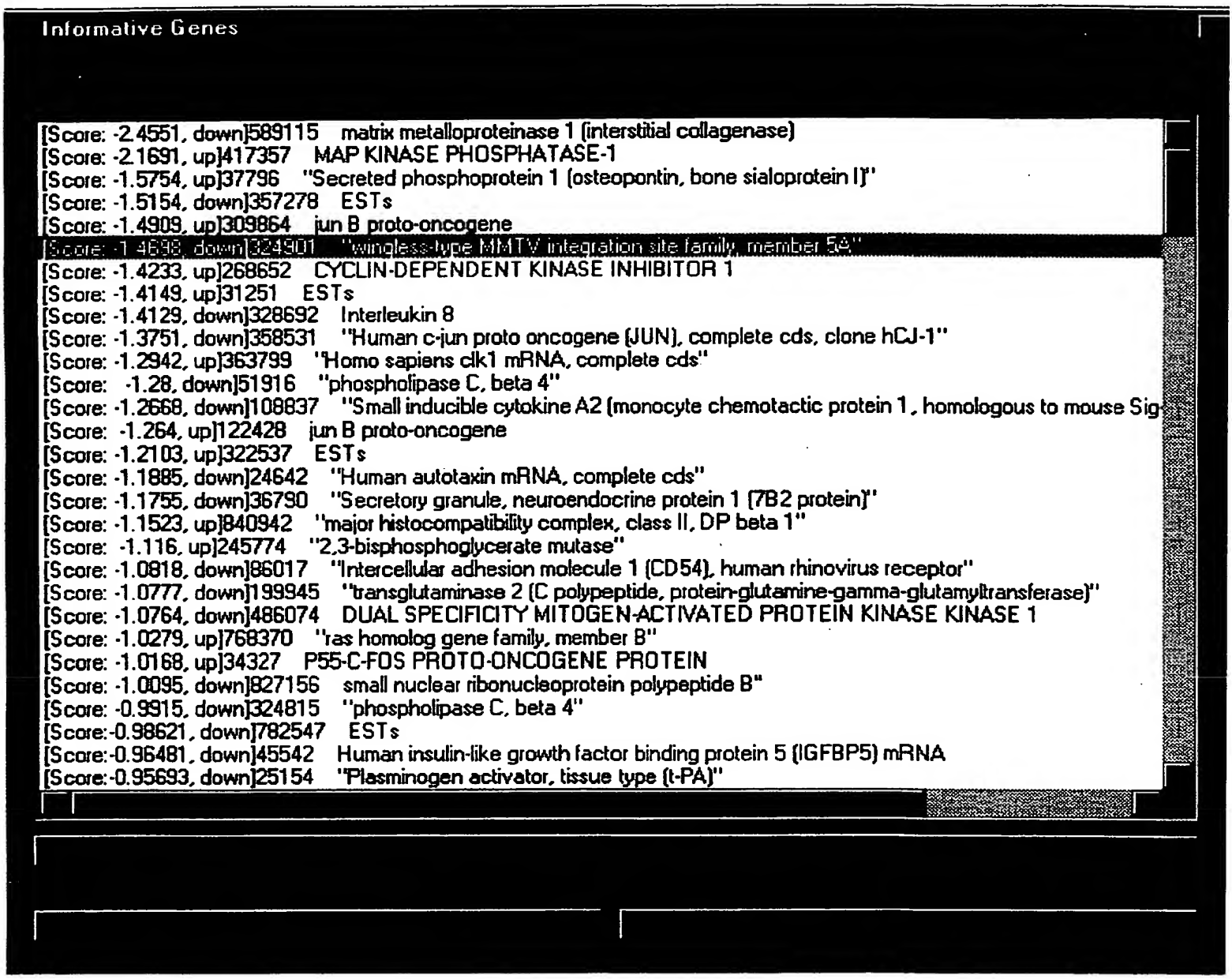


Figure 2 A list of genes, ranked according to the difference in the IQRs for the two melanoma classes. IQR = Inter Quartile Range. Large IQR difference indicates that the gene is more narrowly or stably expressed in one class versus the other. WNT5A is 6th from the top and the 3rd amongst genes that are overexpressed in the mobile samples. The figure is a screenshot from BioClassify by Agilent Laboratories.

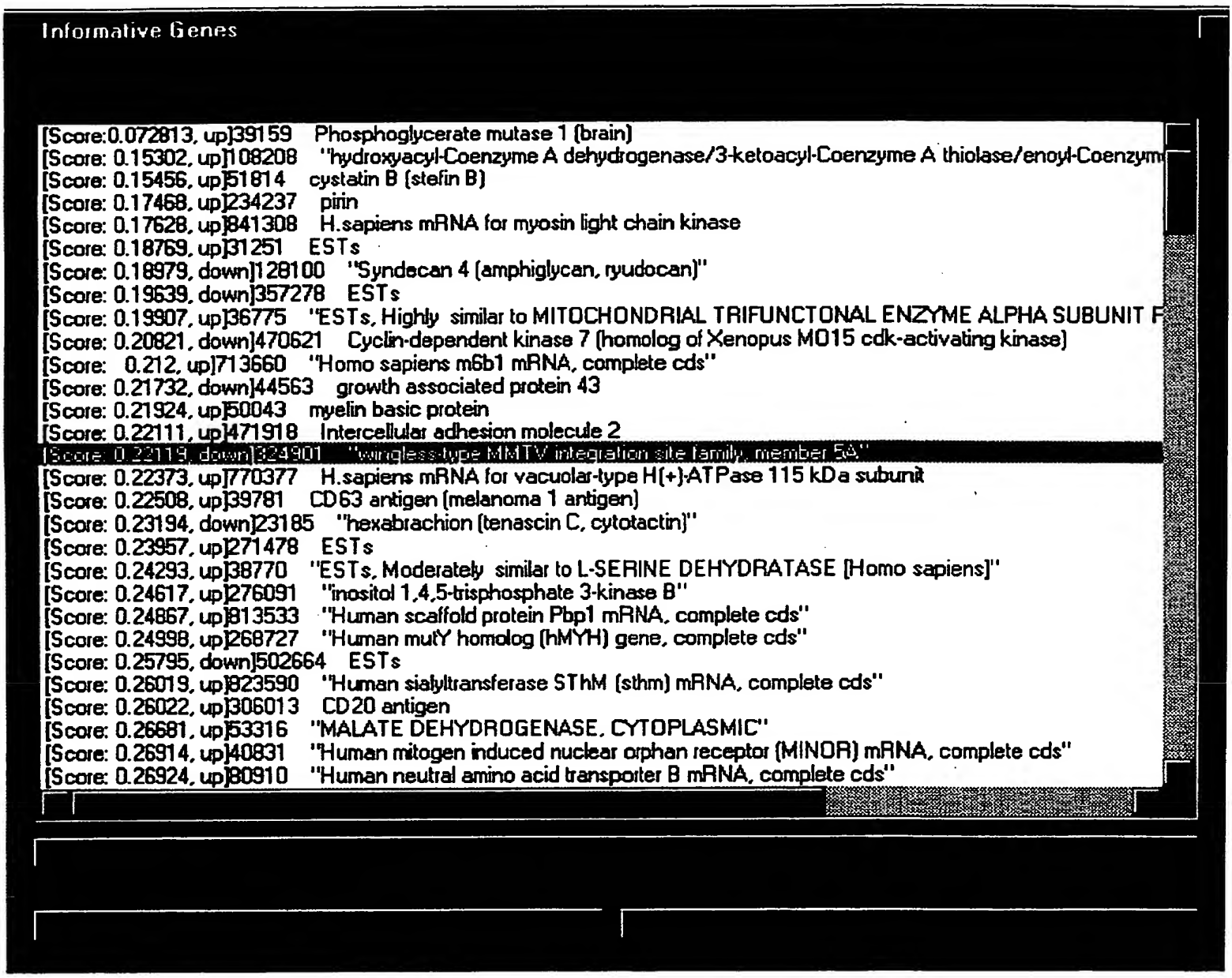


Figure 3. Genes listed according to their Gaussian separation score, a measure of differential expression, beteen the two classes of melanoma. WNT5A is 5th amongst genes overexpressed in mobile samples.